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Progress Report  
Office of Naval Research

"Genetic Regulation in *A. pallida* Symbiosis"  
Grant #: N00014-96-1-0604

This report describes the progress of the project "Genetic Regulation in *A. pallida* Symbiosis". The main goal of the project in year 1 was to identify sequence tags for differentially expressed genes using the SAGE approach.

Since receiving the award from ONR, we concentrated our initial efforts on the zooxanthellae. Isolation of mRNA and cDNA synthesis procedures were selected and tested with yeast and then with a different (non-zooxanthellate) cultured dinoflagellate, *Katodinium rotundatum*. These tests led to an effective protocol. We received cultured zooxanthellae from Dr. Clay Cook, Harbor Branch Oceanographic Institute and proceeded to isolate mRNA and construct cDNA from these samples.

The initial protocol we followed to compare gene expression in cultured and symbiotic zooxanthellae is the one developed for serial analysis of gene expression (SAGE) by Velculescu *et al* (1995)<sup>1</sup>. When successful, SAGE results in the generation of many short (9-12 base pair) tags concentrated in a single sequence. The plan was then to compare frequency diagrams of the tags derived from the expressed genes of the two groups of zooxanthellae to determine which tags represent differentially expressed genes. Tags from these genes were then to be used as probes against full cDNA (expression) libraries and selected clones were to be sequenced.

We initially tested the SAGE protocol with cDNA generated from *K. rotundatum*. Unfortunately, to date little progress has been made towards meeting our initial goal. The application of the SAGE technique proved technically difficult despite repeated efforts by a skilled technician. We are now focusing on constructing representative cDNA libraries from cultured and symbiotic zooxanthellae and will sequence from both the 3'- and 5'-end approximately 5,000 clones from each library and compare the results. This work will be conducted throughout years 2 and 3. This EST approach, though not exhaustive, will identify a large number of zooxanthellae genes and will establish a reference database for future work. In addition, it will allow the identification of differentially expressed genes.

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<sup>1</sup>V.E. Velculescu, L. Zhang, B. Vogelstein, K.W. Kinzler. 1995. Serial Analysis of Gene Expression. *Science*, 270: 484-487.

The budget for year 1 has not been spent in large part due to the problems with progress on the project. As of February 28, 1997, \$56,930.75 total costs was carried over to year 2. The majority of the carryover is related to materials and supplies. We expect to spend all of the carryover funds from year 1 and all of year 2 funds by the end of the second year.